Releases of *Bracon kirkpatricki* (Wilkinson) and *Chelonus blackburni* Cameron for Pink Bollworm Control in Arizona

Production Research Report No. 150

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE
In Cooperation With
Arizona Agricultural Experiment Station

ACKNOWLEDGMENTS

The staff of the Cotton Insects Biological Control Laboratory at Tucson, Ariz., expresses its sincere appreciation to the following persons: Robert G. Buckelew and Rudolph G. Buckelew for allowing the experimentation in their cotton-production fields; Jack Combs, cotton insect infestation consultant, for use of insect population data from nonexperimental fields on the Buckelew ranch; H. M. Graham, Entomology Research Center, Southern Region, Agricultural Research Service, for his suggestions and availability as a consultant regarding pink bollworm ecology; and Blair Bartlett and Louis Dawson, University of California, Riverside, for providing the original *Chelonus blackburni* material from which the culture was started.

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Washington, D.C. Issued June 1973

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402
Price 30 cents domestic postpaid or 20 cents GPO Bookstore
Stock Number 0100–02800

Releases of *Bracon kirkpatricki* (Wilkinson) and *Chelonus blackburni* Cameron for Pink Bollworm Control in Arizona

By D. E. Bryan, R. E. Fye, C. G. Jackson, and R. Patana, *Western Region, Agricultural Research Service, U.S. Department of Agriculture, Tucson, Ariz.*

SUMMARY

From June 10 to September 2, 1971, more than 2 million *Bracon kirkpatricki* (Wilkinson) and 280,000 *Chelonus blackburni* Cameron parasites were introduced into 113 acres of cotton about 20 miles southwest of Tucson, Ariz., to control the pink bollworm (*Pectinophora gossypiella* (Saunders)). During this period the study fields received one application of insecticide for lygus bugs on July 27 and one for the pink bollworms on September 5. In contrast, 120 acres of cotton in the nonrelease area received four insecticide applications for pink bollworm control and one application to control the bollwarm (*Heliothis zea* (Boddie)). Moth captures in sex lure traps were generally lower in the release area than in the nonrelease area. Parasitization by *B. kirkpatricki* ranged up to 25 percent, but this was considered an underestimation. *C. blackburni* was not an effective parasite at the very low levels released.

INTRODUCTION

The pink bollworm (*Pectinophora gossypiella* (Saunders)) is one of the most important pests of cotton in Arizona, California, and New Mexico. Production loss and cost of treatment for pink bollworms have been estimated as high as $15 million per year and may eventually limit western irrigated-cotton culture.

Insecticide treatments for pink bollworm control have intensified other insect pest problems. In parts of Arizona and California the cotton leaf-perforator (*Bucculatrix thurberiella* Busck) has appeared in greater numbers earlier in the season and proved more damaging than previously. Nonchemical control, including insect biological control agents, would appear to be a favorable alternative provided its effectiveness can be demonstrated.

For several years the staff of the Cotton Insects Biological Control Laboratory, Tucson, Ariz., has been accumulating information on exotic and native parasites of the pink bollworm. The usual procedure after acquiring a parasite species includes detailed life-history studies in the laboratory followed by greenhouse tests, field tests in plastic screen-covered cages, 6 by 12 by 24 feet, and finally limited releases in the open field. From June 10 to September 2, 1971, *Bracon kirkpatricki* (Wilkinson) and *Chelonus blackburni* Cameron were released over 113 acres of privately owned cotton about 20 miles southwest of Tucson.

Three major areas of responsibility were defined in the overall scheme of the research: (1) Host rearing headed by R. Patana, (2) parasite production and release led by C. G. Jackson, and (3) field evaluation of control headed by R. E. Fye. This report is presented on a similar basis.

HOST REARING

The beet armyworm (*Spodoptera exigua* (Hübner)) and the pink bollworm were used as hosts to produce parasites for field release. Both species were reared on the limabean diet, as reported by Patana (*17*).[1] The batch of diet was increased to an equivalent of eight times the 1-gallon amount.

Beet armyworm larvae were used as hosts for producing *B. kirkpatricki*. The larvae were reared in clear plastic sweater boxes, 3½ by 10½ by 13½ inches. Hot diet was poured around the sides of the boxes and the bottom was covered with about one-fourth inch of diet by means of an air-pressure dispenser described by Patana (*16*). Approximately 500 newly hatched larvae were implanted per box, and a three-ply wiping tissue was then placed over the box and covered with the lid. When held at about 86° F., the larvae were of suitable size for parasitization in 7 days. During June and mid-July approximately 100,000 larvae per week were used for this purpose.

The laboratory pink bollworm culture was used to provide eggs for rearing *C. blackburni*. A somewhat different method of presenting the diet to the pink bollworm larvae was developed at this laboratory. The diet was spread on sheets of waxed paper rolled together, resembling a jellyroll. This roll was cut into 4½-inch sections and inserted into 1-gallon cartons. These alternate layers of paper and diet, which offered some isolation for individual larvae, thus reduced cannibalism.

The pink bollworm eggs, which were oviposited on 11-cm. filter papers, were placed in the cartons after being held at about 84° F. in the laboratory for 3 days. At this temperature they normally hatched after 4 days. A piece of fiberglass honeycomb material, 6 by 5¼ by 2 inches, with ¾-inch cells was placed on top of the roll of paper and diet in the carton. The eggs were placed on this and the carton was covered with two thicknesses of facial tissue and the lid. The carton was inverted and placed on a rack while the eggs were hatching and during larval development. In 11 days at about 84° the larvae began to cut out of the cartons.

From July 26 through August 23, 1¾ million eggs, on an average, were produced weekly for parasite rearing and for maintaining the parent culture. During this period 59,000 pupae, on an average, were produced weekly for the parent culture.

PARASITE PRODUCTION

Bracon kirkpatricki

Previous Work.—*Bracon kirkpatricki* was first observed as an effective parasite of the pink bollworm in Kenya, Africa, by T. W. Kirkpatrick (*11*). His report prompted requests for the parasite to be sent to Egypt (Anderson (*1*)), Barbados (Bedford (*3*)), and the United States (Noble and Hunt (*14*)). Shipments were received at the former Bureau of Entomology and Plant Quarantine laboratory at Presidio, Tex. *B. kirkpatricki* was propagated there and released in 1935–36 in Puerto Rico, in the Presidio area, and in northern Mexico (Rude (*18*)). In the last two areas some parasites were recovered from parasitized pink bollworms, but the parasites failed to overwinter and releases were discontinued.

In 1968 the Cotton Insects Biological Control Laboratory obtained rearing stock from the Boll Weevil Research Laboratory, State College, Miss., for pink bollworm control in Arizona. Since then laboratory experiments determined that *B. kirkpatricki* could develop on pink bollworms at the high temperatures present in Arizona cottonfields. Greenhouse and field-cage tests demonstrated its potential as a parasite of pink bollworms early in the season when they are in the cotton blooms (Bryan et al. (*4*)).

General Biology.—Adult female parasites are attracted to the host by its movement and possibly by a chemical attractant present in fresh frass. The parasites will not attack a naked larva, but when the host is separated from the parasite by some physical barrier such as a bloom petal, it is then stung and paralyzed. Once the host is paralyzed, the female deposits one or more eggs on or near it.

The noncannibalistic parasite larvae feed externally by sucking body fluids through lacerations made with their mandibles. They are not

[1] Italic numbers in parentheses refer to Literature Cited, p. 21.

attached to any one place but may move to feed at a new spot. The fourth-instar larva stops feeding and finds a place where it is between two closely adjacent surfaces. Here it spins a cocoon and discharges the meconium just prior to the last molt and pupation.

The adults emerge by cutting a hole through the cocoons and usually through one of the adjacent surfaces. Mating occurs shortly after emergence and oviposition begins about 4 days later at 77°–86° F. and may continue for as long as 90 days in the laboratory. More detailed biological studies have been reported by Azab et al. (*2*), Cross et al. (*6*), and Bryan et al. (*4*).

Rearing Procedures.—Several workers have described methods for rearing *B. kirkpatricki* (Noble and Hunt (*15*), Azab et al. (*2*), Cross et al. (*6*), Bryan et al. (*5*)). An improved procedure was described by Bryan et al. (*4*). A modification of this method was employed in this study.

In this procedure 1,500–3,000 parasite wasps were contained in a clear plastic shoebox covered with organdy (fig. 1.). A 6-dram screw-top vial containing a 10-percent solution of levulose and water fitted with a sponge wick (Stoner and Bryan (*19*)) and inserted in a hole on top of the cage served as a source of food. The vials were changed twice weekly.

This cage rested on a piece of rigid plastic on which were layered a sheet of brown paper toweling next to the plastic and about 70 fifth-instar beet armyworms, which were covered with a single thickness of white kitchen toweling (fig. 1). After 30 minutes to 2 hours of oviposition, depending on the age of the female wasps, the cage was removed and the paper towels containing the parasitized larvae were stacked 20 deep with a piece of ¼-inch mesh wire screen separating every fourth assembly. This held the sheets immobile and allowed some air movement between the layers. These were

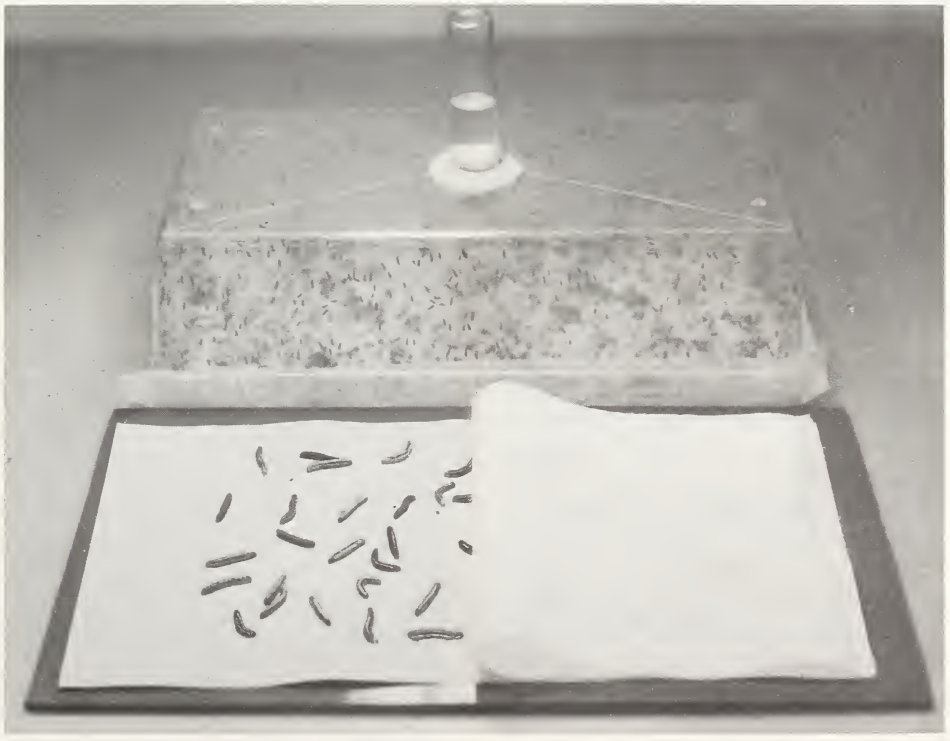

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FIGURE 1.—*B. kirkpatricki* oviposition assembly with cage removed and toweling folded back to show paralyzed beet armyworms.

held for 6–7 days at 86° F. or until the parasites had formed cocoons and in doing so had tied the sheets of toweling together.

The beet armyworms used as host material were dumped, along with their frass, onto a piece of 14 by 18 mesh aluminum wire fabric, suspended over a sink fitted with a garbage disposal. A kitchen sink-type dish sprayer was used to wash the frass through the screen, leaving the clean larvae. The larvae were transferred to a sweater box with a screened bottom and covered with a screened lid. This box was placed in front of a fan until the larvae were partially dry, then they were spread on the sheets of toweling for parasitization.

The sheets were hung vertically in eight darkened chambers fitted on top with inverted plastic funnels. The emergence chambers were built of wood, and each of the eight compartments was fitted with nine rods (three tiers of three) (fig. 2), accommodating 225 sheets per compartment. The parasite adults are positively phototropic and were attracted from the chambers into new parasitization cages by fluorescent lights suspended above the funnels (fig. 3).

When the parasites were collected for release, boxes were substituted for parasitization cages on the funnels. The release containers consisted of cardboard boxes, 15 by 12 by 10 inches, with the flaps removed from one end and the opening covered with organdy. Holes were cut in the side for the feeder vials, and additional sponges were placed on the organdy and kept moistened with the levulose solution. Three pieces of cardboard, 12 by 6 inches, were glued edgewise to the bottom inside the cage to increase the surface area. Such cages contained approximately 20,000–30,000 adult parasites. The boxes of newly emerged adults were held at about 86° F. for 4–7 days before the parasites were released.

Numbers Produced and Rearing Efficiency.—After the adult parasites had emerged from the paper toweling sheets, a 10-percent random sample of the sheets was taken and the empty cocoons were counted. An average production per sheet was obtained and the daily total production was estimated. Table 1 shows the average production per sheet and the total weekly production during the field releases. Production of *B. kirkpatricki* totaled 2,235,400 during the 10-week release period or about 223,500 each week, slightly less than half of the 500,000 goal per week. During the 10 weeks 12,832 sheets of host larvae were parasitized so that 174.2 parasites, on an average, were produced for each sheet. Since there were about 70 host larvae for each sheet, approximately 898,000 larvae were parasitized and each produced an average of 2.49 parasite adults.

Chelonus blackburni

Previous Work.—*Chelonus blackburni* was imported during 1932–36, along with several other parasites including *Bracon kirkpatricki.* It was

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FIGURE 2.—*B. kirkpatricki* adult emergence chamber showing three tiers of sheets containing parasites.

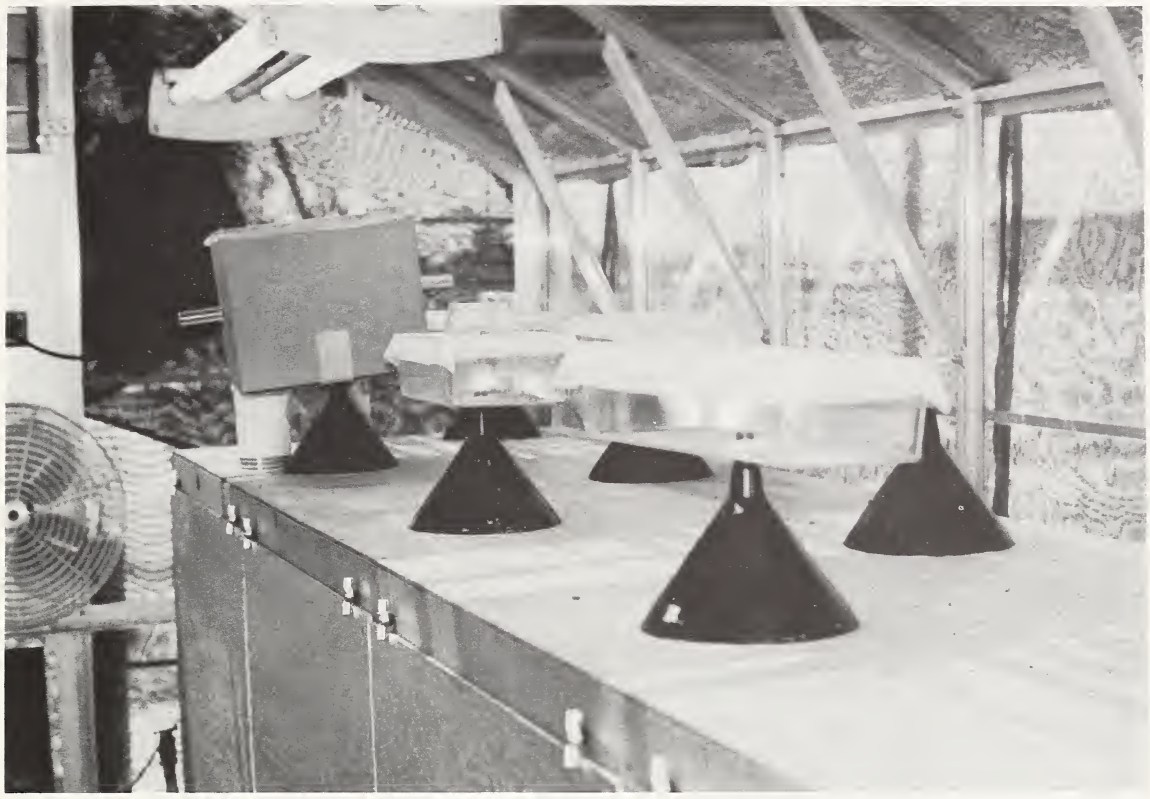

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FIGURE 3.—Top of *B. kirkpatricki* emergence chambers showing parasitization cages and a field release box each positioned on a funnel.

received from Hawaii and propagated at the laboratory at Presidio, Tex. The principal liberations were made in 1936 in Texas, northern Mexico, and Puerto Rico (Noble and Hunt (*14*)). Recoveries were made from parasitized pink bollworms the year the releases were made, but no recovery was obtained in subsequent years. The ability to overwinter in the release areas was not verified, but laboratory data at Presidio suggested that it was possible. In spite of the failure of *C. blackburni* to establish itself, Rude (*18*) considered that of all the imported parasites released in northern Mexico, this parasite had the greatest potential for pink bollworm control.

The culture at the Tucson, Ariz., laboratory was started in July 1970 from 75 females received from Blair Bartlett and Louis Dawson at the University of California, Riverside. The origin of their culture was Hawaii, but *C. blackburni* may also be found in Texas (Muesebeck et al. (*13*)). Unpublished data from this laboratory showed that *C. blackburni* could parasitize bollworms (*Heliothis zea* (Boddie)), tobacco budworms (*H. virescens* (F.)), beet armyworms, and cabbage loopers (*Trichoplusia ni* (Hübner)) as well as pink bollworms. Releases of pink bollworm moths followed by *C. blackburni* in small cages containing potted cotton plants in a greenhouse demonstrated the ability of the parasite to find pink bollworm eggs under the calyx of squares and bolls.

General Biology.—No extensive biological study has been done on this species of *Chelonus*. The biology reported here consists of general observations only. *C. blackburni* is an egg-larval parasite as are others in the genus. The adult female locates a host egg by running her antennae

TABLE 1.—*Beet armyworm larvae parasitized and* Bracon kirkpatricki *adults produced during 10-week field releases, 1971*

Date	Sheets of host larvae parasitized	Average parasite adults per sheet	Total parasite adults produced
	Number	*Number*	*Number*
June 4–10	808	324. 6	262, 300
11–17	1, 263	289. 0	356, 000
18–24	1, 296	233. 0	302, 000
June 25–July 1	1, 278	124. 7	159, 400
July 2–8	1, 808	157. 8	285, 300
9–15	1, 639	79. 3	130, 000
16–22	1, 358	145. 1	197, 000
23–29	1, 165	149. 4	174, 100
July 30–Aug. 5	1, 203	172. 6	207, 600
Aug. 6–12	1, 014	150. 6	152, 700
Total or average	12, 832	174. 2	2, 235, 400

over it. She then positions herself over it and bends the abdomen down and forward to oviposit. In most instances oviposition requires only a few seconds.

One larva of *C. blackburni* develops inside each parasitized host larva until it reaches its last instar. This stage larva cuts out of the host and finishes devouring it externally. It then spins a cocoon, usually adjacent to the carcass of the consumed host. The host larva has reached the last instar before the parasite cuts out of it, and many times the host has formed a death cell. The parasitized host is considerably smaller than an unparasitized host of the same age.

After the cocoon is finished, the parasite becomes a prepupa and discharges its meconium just prior to pupation. The adult emerges through a ragged hole cut in the anterior part of the cocoon. The parasites in these studies were from a uniparental or all female strain. This eliminated any mating and sex ratio problems that might have arisen. The female is ready to oviposit within 1 or 2 days after emergence. The adult parasites ovipositied for approximately 15–20 days in the laboratory at 86° F. and a 14-hour photoperiod.

Rearing Procedures.—Noble and Hunt (*15*) described a method of rearing *C. blackburni* on eggs of the Mediterranean flour moth (*Anagasta* (=*Ephestia*) *kuehniella* (Zeller)). The use of the alternate host was necessary because of the difficulty and expense of rearing pink bollworms at that time. We attempted to utilize their methods, except replacing the flour moth with the Indian meal moth (*Plodia interpunctella* (Hübner)), but we were not successful because of poor and inconsistent egg hatch. The development of the pink bollworm rearing methods, described under "Host Rearing," allowed us to switch to this host, the primary target of these tests.

At first 300–400 adult female parasites were held in cages made from 1-gallon cardboard liquid food containers, 6½ inches in diameter and 6¾ inches long. The center of the lid was cut out and nylon organdy was glued over the hole to provide a removable door to the cage. The bottom of the cage was also removed and a piece of organdy was held in place over it by the rim of a second lid.

As the culture increased, cardboard boxes, 15 by 12 by 10 inches (fig. 4), containing 2,000–3,000 parasites replaced the gallon cartons. The flaps were removed from one end and the opening was covered with organdy. The box was set on its side so that the screened end became the back of the cage. The flaps on the other end were closed and sealed with glue. Then two holes of slightly smaller diameter than the gallon cartons were cut in this end. Two 1-gallon cartons were cut in half and

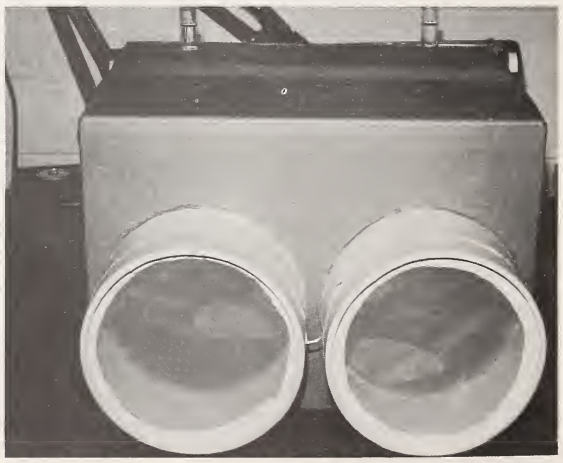

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FIGURE 4.—*C. blackburni* parasitization box showing openings designed to fit those in the emergence chamber.

glued over the holes and capped with screened lids as described for the 1-gallon cages.

Pink bollworm eggs were received from the host-production facility either on 9-cm. filter papers with an average of 800 eggs per paper or on sheets of mimeograph paper, 8½ by 11 inches, with an average of about 4,000 eggs per sheet. One or two filter papers were exposed to the parasites in the 1-gallon cages, and eight to 20 filter papers or one to four mimeograph sheets were exposed in the cardboard box cages for 24 hours in a room maintained at 86° F. and 30–40 percent relative humidity. The number of filter papers or mimeograph sheets of eggs exposed in a particular cage depended on the age and number of parasites in the cage.

The large cages were placed on metal racks so that daylight fluorescent lights attached to each shelf on one side of the rack could shine through the screened backs of the cages. The egg papers were suspended from hooks on a wire that crossed the back of each cage in front of the lights. The positive phototropism characteristic of the parasites then attracted them toward the light and thus to the egg papers. The host eggs were between 1 and 24 hours old when presented to the parasites.

After the 24-hour exposure, the eggs were removed and held for 3 days in plastic shoeboxes. Eight filter papers or two mimeograph sheets cut in one-fourth sections (8 quarters) were then put in each 1-gallon rearing carton, as described under "Host Rearing." The cartons were placed on movable racks in a darkened section of the parasite rearing room maintained at 84° F. and 40–60 percent relative humidity. The host eggs hatched on the next day. About 11 days later the parasitized pink bollworms began cutting out of the cartons. They dropped from the cartons into cardboard boxes containing tissue and strips of cardboard bundled together and set on edge. The larvae formed cells in the cardboard or in the tissue. The last-instar parasite larvae emerged from the host larvae and pupated in the same cells.

The pink bollworm larvae were allowed to cut out of the cartons for 5–7 days. At this point the cartons were opened and the diet rolls removed. The cocoons formed on the tissue, honeycomb material, and lid from host larvae that did not cut out were placed in cardboard boxes. These, along with the boxes that had caught the cut-outs, and the unenclosed diet rolls were put on racks in a dark emergence room, 8 by 8 by 6½ feet (fig. 5).

This room had six holes, slightly smaller in diameter than those of the 1-gallon cartons, cut in one side. The holes were covered with 14 by 16 mesh aluminum wire fabric. Then two lids from the gallon cartons were glued over the holes, facing outward so that the gallon cartons on the parasitization boxes could be fitted into the lids. These boxes rested on a shelf mounted just below the holes. A blacklight mounted on a movable rack was directed through the boxes and the holes into the dark room. As the adult parasites emerged, they were attracted to the blacklight and were caught in the parasitization cages. The screen served to separate the parasites from the moths produced by unparasitized host larvae. The cages were provided with water and ground-up raisins that were moistened daily. The adult parasites emerged over a period of about 10 days; most

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FIGURE 5.—Cartons containing *C. blackburni* cocoons on rack in emergence room.

emerged on the 26th–29th days after the egg sheets were placed in the cartons or on the 25th–28th days after the host eggs hatched.

The parasites to be released were collected in the same types of parasitization cages and in the same manner as those to be used to replenish the laboratory culture. Those to be released were held in the rearing room for 2–4 days.

Numbers Produced and Rearing Efficiency.— The production of *C. blackburni* gradually increased through the season as more host eggs became available and rearing procedures improved. The switch of host material from the Indian meal moth to pink bollworms was not anticipated and there was a delay as the pink bollworm culture was increased and rearing methods were worked out on the new host.

The largest numbers of host eggs were received for parasitization after the middle of July and no actual production data were kept prior to this time. However, the numbers of filter papers and mimeograph sheets of eggs were recorded from July 21 to August 24. Samples of the egg papers were taken and the eggs on each paper were counted. From these counts, averages of 800 eggs per filter paper and 4,000 per mimeograph sheet were obtained. Thus the total number of eggs exposed each day could be estimated. The parasite adults resulting from parasitization of these eggs emerged from August 19 through September 22. Daily adult parasite emergence was estimated by counting the number of dead parasites remaining in some of the parasitization boxes just before discarding them.

During the 35-day period 6,855,000 eggs were exposed to the parasites and 377,000 adult parasites were produced or, on an average, 195,857 eggs were exposed per day and 10,771 parasites were obtained. These data indicate that 5.5 percent of the eggs exposed for parasitization produced adult parasites.

FIELD SITE

The tests were conducted at Robles Junction, Ariz., about 20 miles southwest of Tucson on a ranch comprising about 1,000 acres of tillable land (fig. 6). The usual cropping system included cotton, grain sorghum, and small grains. About one-third of the ranch is separated from the remainder by Brawley Wash, which drains a large area south of the study site. The study fields included F3, F5, F6E, F6S, F6W, and F7.

The parasites were introduced into 113 acres of cotton (fields F3 and F5) on one side of the wash. The check field (F6E) was an 81.5-acre field on the other side of the wash about 200 yards from the test fields. In addition to the check field, about 120 acres of cotton were grown in three adjacent fields (F6W, F6S, F7). When 365 acres of small grain were harvested in June, about 300 acres of grain sorghum were planted. The crops on the ranch were well managed with the best agronomic practices.

On the opposite side of the highway from the study area is a ranch of similar size farmed for alfalfa hay production with some wet pasture areas included. The two ranches are separated by 6–7 air miles from the nearest agricultural land.

FIELD METHODS

Parasite Introductions.—The parasites were transported from the laboratory to the field in a van equipped with two generator-powered air conditioners. This allowed for continuous cooling even when the van was not moving. A schedule of two releases each week on Mondays and Thursdays was followed, except when postponements were necessary because of rain or insecticide application. Releases were made at dusk to allow a period of acclimatization for the parasites prior to the heat of the ensuing day.

The parasites were released in five locations in field F3 and three locations in field F5 (fig. 6). The nylon organdy front of the cages was slit and the parasites were distributed on the cotton plants in the immediate vicinity. The *B. kirkpatricki* frequently flew several yards before settling into the cotton rows. The *C. blackburni* departed in short, looping flight. As soon as they settled, they started searching the surfaces of the cotton plants.

White Bloom and Insect Count.—To determine the infestation and the parasitization of pink boll-

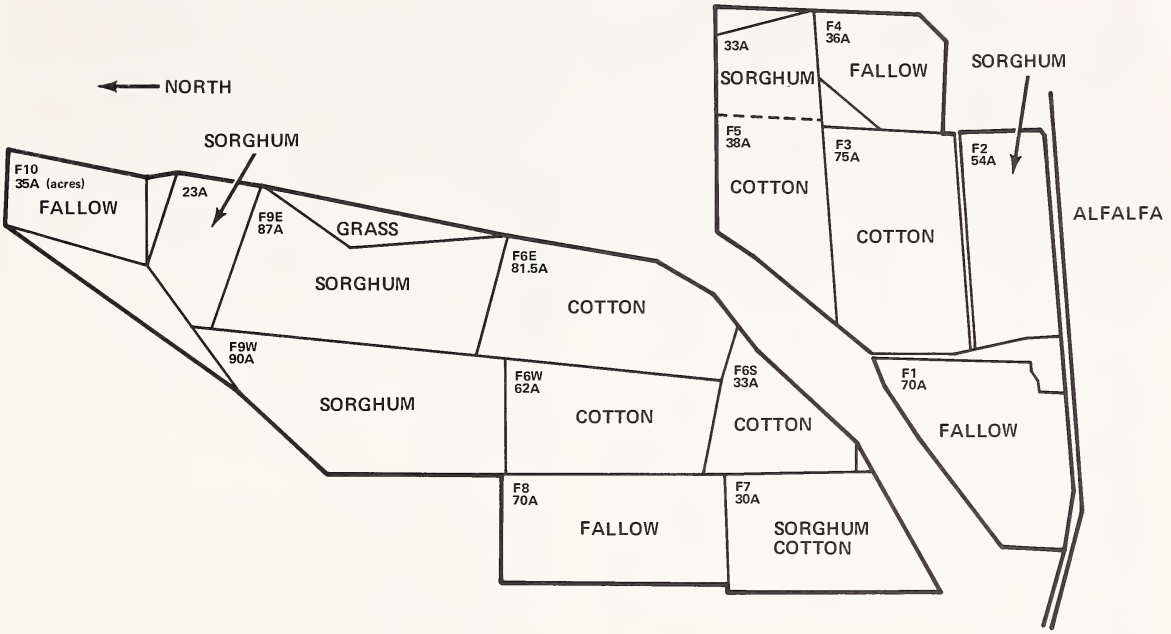

FIGURE 6.—Diagram of fields and crops on Buckelew ranch, Robles Junction, Ariz.

worms in the white blooms, the blooms on several thousand feet of row were inspected twice weekly except when irrigation and rains did not permit entry into the fields. As five or six workers passed through the field, the cotton in the rows on either side was parted and the number of white blossoms counted. If a rosetted bloom was noted, it was removed from the plant and placed in a 1-ounce creamer cup and taken to the laboratory for inspection. The number of blossoms rosetted was noted and the number of paralyzed or parasitized pink bollworms was recorded. From these data the number of white blooms, the number of pink bollworms in the blooms, and the number of parasitized or paralyzed pink bollworm larvae per acre were calculated.

In addition to the pink bollworms, bollworms and beet armyworms were similarly collected and inspected. The larvae of all three species were held until pupation or until the naturally occurring parasites emerged.

The limabeam medium described by Patana (*17*) was used as the holding medium.

Boll Collections.—During the boll-production period 600 vulnerable bolls from each of the study and check fields were collected twice weekly. The samples of bolls were reduced in replicates containing 50 bolls. One set of four replicates was inspected immediately for the presence of pink bollworms and their exit holes. A second set of four replicates was retained for 2–3 weeks in a screened insectary in Tucson and then inspected for the presence of pink bollworms and their exit holes. A third set of four replicates was retained to overwinter in the insectary to determine the presence of diapausing larvae that might harbor either the introduced species or naturally occurring parasites. The bolls were placed in a hardware cloth basket in the upper part of plastic sweater boxes with screened ventilating holes in the bottom and cover. A crumpled paper napkin was put in the bottom of the box for a pupation site of the emerging larvae.

Plant Counts.—At weekly intervals 100 randomly selected sample points were inspected in each of the study and check fields. Early in the season five plants were inspected, but late in the season one plant was examined at each of the 100

points. During the inspection the squares, blooms, bolls, common predators, and other major cotton pests were counted. On three inspections the plants for a single foot of row were counted at the 100 randomly selected points in the field. From these data the plant densities in the field were calculated and the fruits or insects per acre were estimated.

Pink Bollworm Adult Populations.—Traps (fig. 7) for male adult moths with Hexalure as the attractant were installed on March 18, 1970. From then until May 20 three sets of eight traps each were in use and on May 20 two additional sets of eight traps each were installed. Before the cotton plants emerged, the traps were placed along the edges of the field about 3 feet above the ground and approximately 50 feet apart. When the plants emerged from the ground, the traps were moved to border rows that divided the field into blocks to prevent heavy rainfall from washing out the plants. The traps were checked twice weekly during and once a week before and after the growing season.

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FIGURE 7.—Hexalure trap for pink bollworm males.

The traps consisted of a 7-ounce plastic cup [2] with the bottom removed. It was glued to the inverted tapered part of a 14-ounce Styrofoam cup, the bottom of which was closed with the regular cup cover.[3] The Hexalure was placed on a small piece of dental roll in a wire clip in the mouth of the upper tapered cup. A piece of Vapona strip [4] was put in the lower part of the trap as a killing agent. A strip of fibrous tape was folded over the upper edge of the cup to prevent breakage of the trap when attached to a rod standard, one-fourth or nine-thirty-seconds inch, with a pinch-type paper clip. The traps were rebaited with 25 mg. of Hexalure at 2-week intervals. The killing agent was replaced at 2- to 3-week intervals.

Bioclimatological Data.—During the growing season the temperature, relative humidity, rainfall, windspeed, and wind direction were recorded in the study area.

Loss Estimates.—One to three days before the first picking of cotton in each field, the losses due to the pink bollworm and the cotton bollworm were estimated. Two hundred plants in each of the production fields were examined and the number of green bolls, undamaged dry bolls, and bolls with from one to five locules damaged by the pink bollworm or the bollworm were recorded.

Toxicity of Insecticides.—At 1-day intervals after the application of insecticides in field F3 on July 27 and in field F6E on August 21 (table 2), leaves were excised from the plants in the sprayed fields. The leaves were placed in plastic bags in Styrofoam portable ice chests and removed to the laboratory. There the leaves were put in vials of water installed in tapered cage bases and covered with 1-quart squat ice cream-carton cages, which had one end covered with nylon organdy. Ten replicates of 10 parasites each were introduced onto the leaves and held for 24 hours in an air-cooled greenhouse. Air temperatures in the field and in the greenhouse were recorded during the two tests.

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[2] Solo Cup Co., Chicago, Ill., Cozy Cup.

[3] Thompson Industries, Phoenix, Ariz. Styrocup and Styrolids.

[4] Shell Pest Free Strip.

TABLE 2.—*Insecticide applications for cotton pests, Robles Junction, Ariz., 1971*

Date	Field	Material	Rate per acre	Target insect
		Pounds per gallon	*Gallons*	
July 23	F6S, F6W, F7	Ethyl parathion 3+toxaphene 6	0.5	Pink bollworms.
27	F3, F5	do	.33	*Lygus* spp.
30	F6S, F6W, F7	do	.33	Pink bollworms.
Aug. 6	F6S, F6W, F7	do	.33	Do.
21	F6S, F6E, F6W, F7	Toxaphene 8+methyl parathion 2	.5	Bollworms.
Sept. 5	F6S, F6W, F7, F3	do	.5	Pink bollworms.

RESULTS AND DISCUSSION

Parasite Introductions.—The numbers of *B. kirkpatricki* and *C. blackburni* introduced into the 113 acres of cotton are presented in table 3. More than 2 million *B. kirkpatricki* parasites were introduced between June 10 and August 12 and about 283,000 *C. blackburni* between June 17 and September 2, with most of the *C. blackburni* released after July 20.

***Suppression of Host Population in Blooms by* B. kirkpatricki.**—The data in figure 8 show some suppression of the pink bollworm population in the blooms during the blooming period in fields F3 and F5 as compared with that in field F6E. The parasitization by *B. kirkpatricki* rose to 25 percent on about July 9 but declined thereafter. The decline on about July 10–15 coincides with the period of highest temperatures during the growing season. During this period the air temperature at midday exceeded 100° F. for 6–8 hours and highs of 106°–108° were attained.

The percent parasitization of the pink bollworms in blooms may be underestimated because at the time of bloom the pink bollworm has been subjected to less than one-half the period of vulnerability to *B. kirkpatricki*. When the white bloom passes into the pink bloom stage and thence into a drying stage, the pink bollworms within the petals remain vulnerable to *B. kirkpatricki*.

The suppression of the pink bollworm population in blooms is further evident in the capture of the adult moths in the Hexalure traps from field F3 (fig. 9). However, the suppression is not evident in the trap catches from fields F6W and F7.

On July 12 the grower on the adjacent ranch cut a major acreage of alfalfa and thereafter allowed the fields to dry out without further irrigations. As a consequence, a relatively large number of *Lygus* adults (table 4) entered fields F3 and F5 and oviposited. By late July the result-

TABLE 3.—*Parasites released, Robles Junction, Ariz., 1971*

Date	*Bracon kirkpatricki*	*Chelonus blackburni*
	Thousands	*Thousands*
June 10	158	----------
14	158	----------
17	205	2
21	180	----------
24	120	2
28	75	----------
July 1	64	----------
5	102	----------
8	145	----------
12	76	2.5
15	54	----------
20	111	2.5
22	86	6.5
Aug. 4	340	45
11	39	8
12	106	42
16	----------	35
19	----------	22
23	----------	30
26	----------	26
Sept. 2	----------	60
Total	2,019	283.5

ing population of *Lygus* nymphs was such that an application of insecticide was made to these two fields for their control on July 27 (table 2). Although this application also controlled the pink bollworms in the field, the flight of moths in field F3 (fig. 9) at that time was not great enough to require their control. At the same time (table 2) fields F6S, F6W, and F7 required insecticides for the pink bollworms and applications were made on July 23, 30, and August 6. Thus the major cotton acreage on the ranch required three applications to control the pink bollworms during this period, whereas the study fields F3 and F5 were subjected to a single application, which was not directed specifically at the pink bollworms but at the *Lygus* populations.

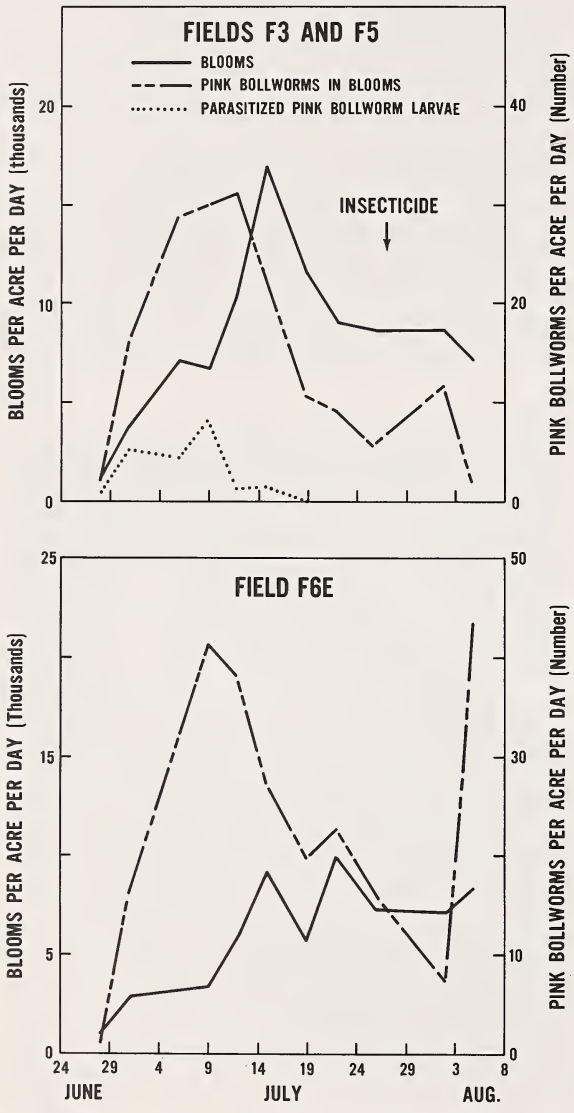

FIGURE 8.—Densities of blooms, pink bollworms in blooms, and pink bollworm larvae parasitized by *B. kirkpatricki* in fields F3 and F5 and densities of blooms and pink bollworms in blooms in field F6E.

Overall Infestation Data and Parasitization by C. blackburni.—The overall infestation data are presented in figure 10. The pink bollworm infestation and the parasite levels based on the various boll collections throughout the season are presented in table 5. At the levels of introduction, as indicated by the parasitization detected in the boll samples, *C. blackburni* had little apparent effect on the pink bollworm populations. These populations in fields F3 and F5 and field F6E were very similar. The population increases were closely associated with the availability of vulnerable (15 to 30 days old) bolls and the flights of adults (figs. 9 and 10). An early peak about August 1 in fields F3 and F5 was associated with the shift of the pink bollworms from the squares to the bolls. The early suppression of a small flight of adults during the previous week with insecticides (fig. 9 and table 2) apparently was responsible for the short-lived surge. Generally the parasitization rate by *Chelonus* was low and little suppression is noted in the data presented in figure 9 for field F7.

Since populations of naturally occurring predators (table 6) and competing populations of other lepidopterous pests of cotton (table 4) were similar, the natural predators would have a similar effect in the three fields and the pest populations would offer similar competitiveness for the action of the parasites. Therefore the predator and pest populations need not be considered in interpreting the action of the parasites.

The effects of high temperatures on longevity, fecundity, development, and egg hatch (Fye and Poole (*9*), Fye and Surber (*10*), Fye and McAda (*8*) were not applicable since only one short period between July 9 and 12 had high temperatures.

Population Assessment Problems.—The vagaries of sampling insect populations are well revealed in the data presented in tables 4–6 and figure 9. Since single insects assume major propor-

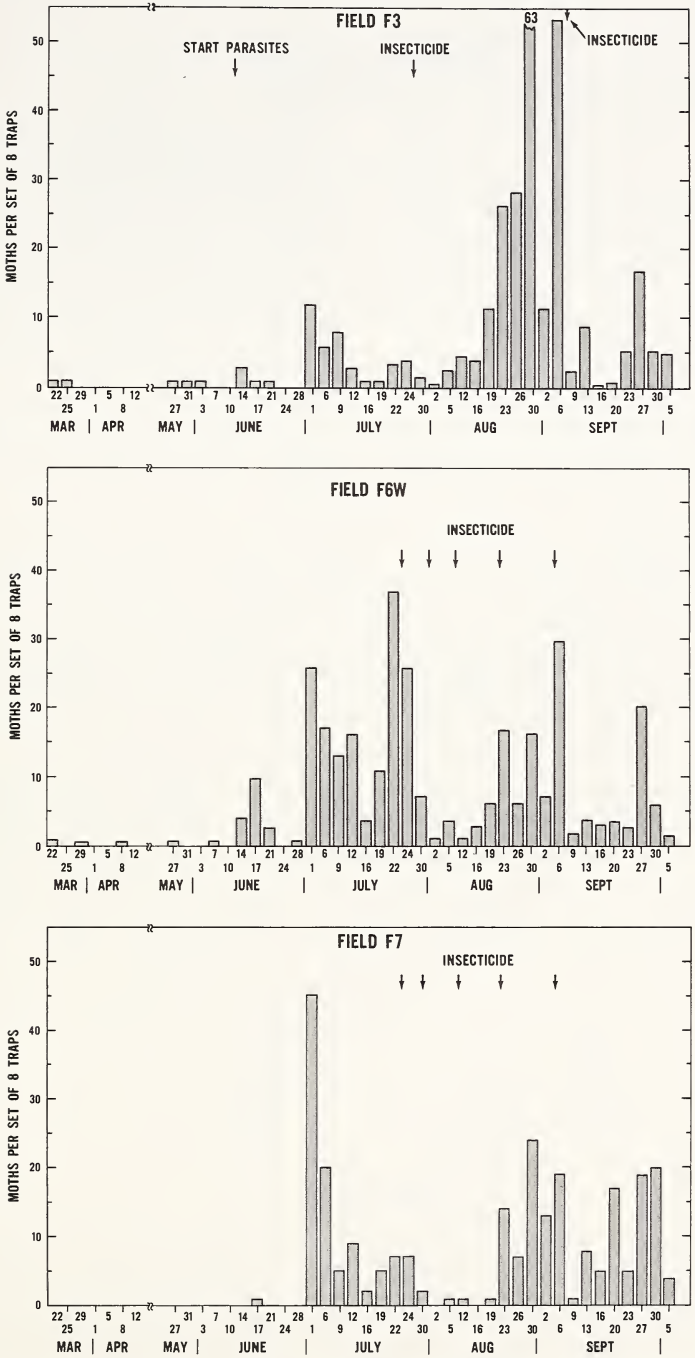

FIGURE 9.—Numbers of pink bollworm moths per set of eight Hexalure traps in fields F3, F6W, and F7.

tion when projected into total populations with calculated conversion factors, a few detected insects may show an apparent difference in the mean populations of the insects, which is in fact unrealistic.

Kuehl and Fye (*12*) showed that the variance associated with the mean populations of most insects associated with cotton is generally as great or greater than the mean itself, and frequently the standard errors of the mean may be relatively large. Therefore what may appear to be major jumps in the population when the mean populations are considered may be sampling errors inherent in the field sampling of populations. Thus if the differences in the populations are to be significant, the differences between the control and test fields must be relatively large and consistent.

Further sampling error is evident in the data concerned with blooms and bolls. The bloom data in figure 8 would indicate a much lower boll potential than the data in figure 10. This is evidence of personal bias by which plants are "randomly" selected by individuals doing the sampling. Fye et al. (*7*) showed that the mean populations on five plants selected at random in the field were always less than five times the mean populations determined from the first plant selected in the series of five plants.

Although there is some possibility of error in white bloom counts in the field because of the

TABLE 4.—*Populations of pest species per acre in fields F3, F5, and F6E during 1971*

Date	Bollworms				Cabbage loopers		Beet armyworms		*Lygus* spp.		Flea-hopper
	Eggs	Larvae	Damage		Eggs	Larvae	Eggs	Larvae	Adults	Nymphs	Nymphs and adults
			Squares	Bolls							
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*
FIELDS F3 AND F5											
June 3								0.5			
10						0.2			0.1	0.2	0.1
17	0.8							.1	.2	.5	.5
24	.9	0.1	0.8			.1	0.1	.1	.2	.1	6.5
28	.2	.1	.1							.1	6.5
July 1	.1	.1	1.5				.1			.4	2.1
13	.5	.9	.5	0.5					2.8	1.8	6.1
20		.9	.5			1.5			.9	3.3	2.4
Aug. 3	8.4	3.7	1.4	3.7	22.1	4.7			4.3	.9	
16		10.3	2.8	4.3	7.1	24.4		.5		1.8	
23		8.4	2.4	1.5		18.8		.9		1.8	
30		1.8	.5	6.5	1.8	.5			2.8		
FIELD F6E											
June 3										.5	
10								.1			.1
17	.1		.1			.1				.6	.6
24	.2	.1	.6			.1		.1	.1		.5
28	.5	.1	1.2					.1	.1		2.6
July 1	.3	.3	1.2			.1		.1		.3	1.4
13		1.5	2.4						1.8	.5	3.3
20		3.3	1.5	2.4		.5		.5	.5	1.8	1.5
Aug. 3	.5	9.9	4.3	2.8	9.0	4.3			1.5	.9	.5
16	.9	8.4	4.3	16.9	23.1	13.1		.5	.5	4.7	
23				10.9		.9					
30		2.8	4.3	11.8	4.3	.9		.5			

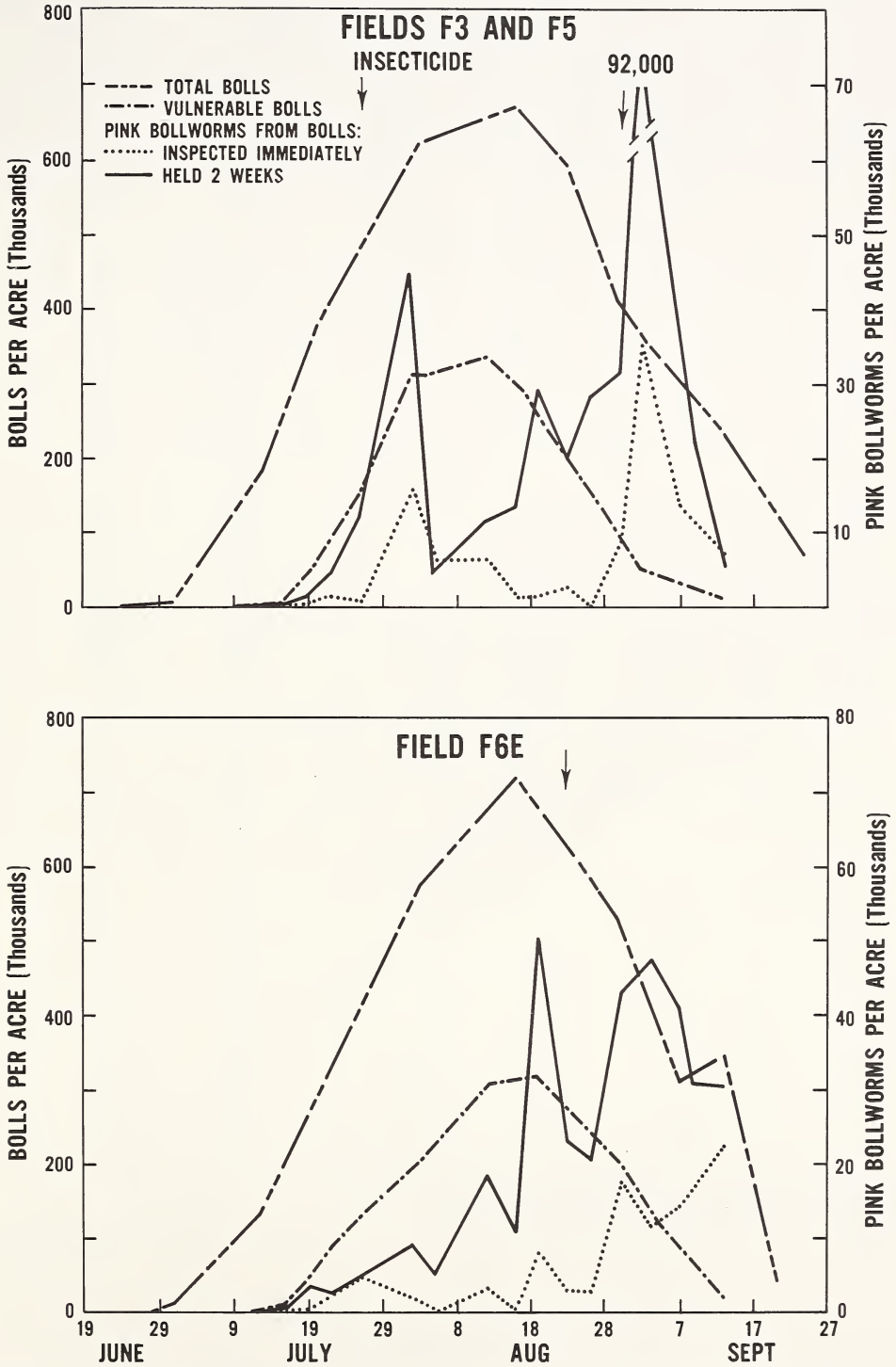

FIGURE 10.—Fruiting pattern and pink bollworm populations in vulnerable bolls from fields F3, F5, and F6E when bolls were inspected immediately and when held 2 weeks.

TABLE 5.—*Pink bollworm infestations based on 200 bolls in fields F3, F5, and F6E, Robles Junction, Ariz., 1971*

Date	Immediate inspection			Inspection after holding 2–3 weeks [1]		
	Pink bollworm larvae	Exit holes	*Chelonus blackburni*	Pink bollworm larvae and pupae [2]	Exit holes	*Chelonus blackburni*
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
FIELDS F3 AND F5						
July 9	0	0	0	0	1	0
12	1	0	0	4	5	0
15	1	0	0	1	0	0
19	2	0	0	6	6	0
22	3	0	0	10	12	0
26	1	0	0	16	18	0
Aug. 2	10	1	1	29	33	2
5	4	1	0	3	4	0
12	4	3	0	7	17	0
16	1	1	0	9	13	1
19	1	2	0	23	36	0
23	4	7	1	20	28	0
26	0	5	0	37	51	0
30	18	15	0	70	94	0
Sept. 2	147	14	6	368	388	17
7	92	21	1+1(?)	225	299	0
9	[3]	[3]	----------	204	286	0
13	207	63	5	155	190	0
FIELD F6E						
July 12	0	0	----------	4	7	----------
15	2	----------	----------	8	9	----------
19	2	1	----------	15	17	----------
22	6	2	----------	7	5	----------
26	7	----------	----------	8	7	----------
Aug. 2	2	4	----------	9	11	----------
5	0	4	----------	5	13	----------
12	2	6	----------	12	15	----------
16	0	2	----------	7	8	----------
19	5	0	----------	32	48	----------
23	2	7	----------	17	21	----------
26	2	9	----------	17	41	----------
30	17	4	1	42	36	----------
Sept. 2	17	7	----------	70	94	----------
7	32	24	----------	90	159	----------
9	[3]	[3]	----------	93	121	----------
13	228	23	1	300	241	----------

[1] 4 replicates of 50 bolls held in insectary for 2–3 weeks.

[2] Larvae from bolls plus pupal cases in holding box.

[3] No count.

heavy foliage at the time of maximum bloom, the bloom count error would have to be much greater than the potential error to account for the number of bolls presented in figure 10. Thus it is evident that the "random" selection of plants in a field by the individual checkers involved a positive bias for plants of greater physical or physiological stature. Therefore an overestimate of the number of bolls in relation to the number of blooms is inevitable in most studies unless a more positive random selection is employed.

The data in figure 10 indicate that the examination of bolls at the time of collection may be misleading in regard to the total pink boll-

TABLE 6.—*Populations of predators per acre in fields F3, F5, and F6E during 1971*

Date	*Chrysopa* spp.			*Collops* adults	Ladybirds [1]	*Nabis* spp. [1]	*Geocoris* spp. [1]	*Orius* spp. [1]	Spiders [1]
	Eggs	Larvae	Adults						
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*
FIELDS F3 AND F5									
May 20				1.4	1.0		1.0	0.5	2.4
27				.5	5.3	0.5	.5	1.0	4.3
June 3	1.9			.5	1.0		.5		4.3
10	.1	0.1	0.1	1.2	2.5	.1	1.0	2.0	1.9
17	2.7	.9	.3	1.9	6.6	.5	2.2	4.3	1.8
24	3.5	.1	.1	3.5	4.7	.1	3.1	5.9	1.5
28	.9	.1	.1	4.3	3.8	.2	3.5	5.2	2.6
July 1	.8	.3	.3	5.2	3.4	.5	3.3	9.0	2.1
13	8.4			14.6	9.0	1.5	3.7	18.8	4.3
20				9.9	5.2		4.7	6.1	5.6
Aug. 3		.5		4.7					.9
16			.5	2.4					.9
23		1.5	.5	.9	.5		.5		.9
30		.5		.5					2.8
FIELD F6E									
May 20				2.4			.5		.5
27				.9	.9				.5
June 3	.5				3.3		.5		2.8
10				.5	.7		.1	.3	1.2
17	1.6	.1	.1	3.5	2.4	.1	1.0	2.7	1.0
24	3.1	.2	.6	4.3	2.0	.1	1.1	2.9	1.3
28	1.5	.6	.5	4.8	3.3	.2	3.4	6.4	.8
July 1	1.8	.9	.3	6.2	2.7	.5	1.8	4.9	1.2
13	9.0	.5	.9	10.3	5.6	.9	2.8	8.4	5.2
20	11.8		.9	8.0	2.4	.5	3.7	6.1	2.4
Aug. 3	2.4		.5	5.2	.5			.5	.5
16	15.0		1.5	2.4				.5	
23	.9	.5							
30	6.5	.9	.5						

[1] Adults + immatures.

worm population present at the time. Slosser [5] demonstrated that bolls held for about 2 weeks had a higher infestation of pink bollworms than bolls examined immediately. This observation is corroborated in the data in figure 10, which show greater pink bollworm populations in the bolls held about 2 weeks as compared with populations detected in a companion set of bolls examined immediately after collection.

It is apparent that the underestimation of the population can be attributed to at least two factors. The first is the presence of eggs on the boll bracts and calyxes when the bolls are picked. These eggs subsequently hatch and develop to a point where they may be detected in the bolls held for at least a short period after collection. The second factor is the potential mortality due to the rapid proliferation of the cells within the boll that may effectively interfere with the establishment of the young pink bollworm larvae. It is not unusual to find a number of small mines in the carpel walls with no associated larvae in the boll. Evidence of proliferation is also frequently observed on the carpel wall near the mines.

The presence of the hatching population at the time of collection is further supported by the data in figure 10, which, when considered with figure 9, show a low moth population when the larval population estimates for the immediately examined and short hold bolls are similar. Since the proliferation in the boll will cease when the boll is excised from the plant, the potential survival of the hatching larvae is greater than the potential survival of larvae hatching on the plant. In addition, the many physical and biological hazards to the hatching eggs and larvae are removed by the more ideal conditions associated with the rearing in the insectary. Therefore depending on the purpose of a population assessment, the inspection either immediately or after a holding period may be more desirable.

Effect of Rainfall on Infestation.—One of the most complicating factors in the study was the rainfall during July and August. The drenching thunderstorms frequently prevented the field assessment crew from entering the fields and therefore several wide spreads occur in the data. More complicating than the sporadic data is the lack of knowledge of the effect of these thunderstorms on the insect population. Rainfall of from 0.5 to 2.5 inches per hour is not uncommon in Arizona thunderstorms, and at least seven storms with this amount of precipitation occurred from July to early September. The rainfall is frequently accompanied by gusty winds and the effect of this combination of factors on the egg and adult stages of the insect complex in cotton is unknown.

Nor is the effect of the rapid rainfall on the pupae in the soil surface and in the trash on the soil surface known. It would be expected that large numbers of the pupae in the soil may be sealed in and the adult moths unable to emerge. It is also possible that the pupae in the trash would be washed from the fields as the runoff occurred. Until these factors can be evaluated the mortality associated with the heavy rainfall cannot be determined.

Response of* B. kirkpatricki *and* C. blackburni *to Insecticides.—During the field evaluation of *B. kirkpatricki* and *C. blackburni*, it was possible to determine the effect of insecticides on the parasites and to determine how long the residual insecticides caused major mortality to the introduced parasites.

The results of the tests are presented in table 7. The parathion-toxaphene mixture used on field F3 resulted in total kill of the parasites for the first 4 days after the application. Major mortality occurred up to the seventh day, when the rainfall apparently reduced the effectiveness of the insecticide for a 24-hour period. However, after this period a mortality of about one-fourth of the parasites occurred. This phenomenon was also observed in the second test and suggests that the rainfall may have removed an oxidized layer from the insecticide droplets on the leaves and thus brought toxic materials into position for contact with the parasites.

The second test in field F6E, which was sprayed with a methyl parathion-toxaphene mixture (table 2), resulted in similar mortalities. Complete mortality occurred for the first 4 days with major mortality for the following 3 days, particularly with *C. blackburni*. The increased effectiveness of

[5] SLOSSER, J. E. POPULATION GROWTH OF THE PINK BOLLWORM, PECTINOPHORA GOSSYPIELLA (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE). 1971 [Unpublished Ph.D. dissertation. Copy on file Dept. of Ent., Univ. of Ariz., Tucson.]

TABLE 7.—*Mortality of* Bracon kirkpatricki *and* Chelonus blackburni *placed on excised leaves from sprayed fields with no prior 1971 insecticide history (10 replications of 10 parasites daily)*

Days after application	Test 1 in field F3 [1]					Test 2 in field F6E [2]				
	Temperatures		Rainfall	Mortality of—		Temperatures		Rainfall	Mortality of—	
	Field	Holding		*Bracon*	*Chelonus*	Field	Holding		*Bracon*	*Chelonus*
	° F.	° F.	*Inches*	*Percent*	*Percent*	° F.	° F.	*Inches*	*Percent*	*Percent*
<1	75–92	69–92		100	100	70–88			([3])	([3])
1	75–104	71–94		100	100	68–88			([3])	([3])
2	70–102	70–94		100	100	68–86	72–92		100	100
3	75–101	70–93	0. 38	100	100	70–92	72–92		100	100
4	69–100	69–92		100	100	71–92	70–93		100	100
5	72–98	70–94	. 05	77	93	67–94	73–94	0. 15	38	85
6	70–96	72–94	. 08	9	53	71–94	71–94		16	27
7	77–100	70–92	1. 50	53	32	70–96	71–88		14	61
8	65–96	71–94		5	8	68–92	72–95		27	59
9	69–89	71–96		21	26	71–98	72–94		13	44
10	71–98	66–91		4	53	70–99	73–94		36	52
11						71–96	69–96	1. 05	4	12
12						67–98	71–94		6	0
13						70–90	72–94		5	18

[1] 1 pound of ethyl parathion +2 pounds of toxaphene per acre.
[2] 1 pound of methyl parathion +4 pounds of toxaphene per acre.
[3] No test.

the insecticide against *C. blackburni* as compared with *B. kirkpatricki* may possibly be attributed to the behavior pattern of the two parasites. The *Bracon* adults tend to be more passive, whereas the *Chelonus* parasites actively search the leaf surfaces and thereby are vulnerable to an increased insecticide dosage. As previously noted, the heavy rain during the second test decreased the mortality of the parasites because of the insecticide application, but after 24 hours the mortality reverted to the prerain level.

These experiments indicate that the insecticides are highly hazardous to the parasites and the insecticide residual action may be such that introductions cannot be resumed for about 10–14 days after the insecticides are applied. Periods such as these during a rapid increase of a target population would negate the potential of the parasites involved.

Bollworms.—The data in figure 11 indicate that the bollworms in fields F3 and F5 were suppressed in a manner similar to the pink bollworms. The suppression may be partially attributed to the presence of *Bracon* and the relatively high parasitization by *Microplitis croceipes* (Cresson). Although high parasitization by *M. croceipes* was present in field F6E, the bollworm population (fig. 11) increased to a level that required insecticides for its suppression (table 2). Again the parasitization by *B. kirkpatricki* on the bollworm is probably underestimated because of the mobility of the larvae and the disregard for populations in parts of the cotton plant other than the white blooms.

Cotton Losses.—The data in table 8 indicate that the pink bollworm, bollworm, and boll rot damage in all the study fields on the ranch was similar. Since the data were taken just prior to the first picking of the cotton, the figures probably reflect the critical damage to the better quality early-season cotton. Although the green bolls at the time of picking harbor a large pink bollworm population (fig. 10), its effect on the lower quality cotton is not so great as would be expected for a large population in the earlier matured cotton.

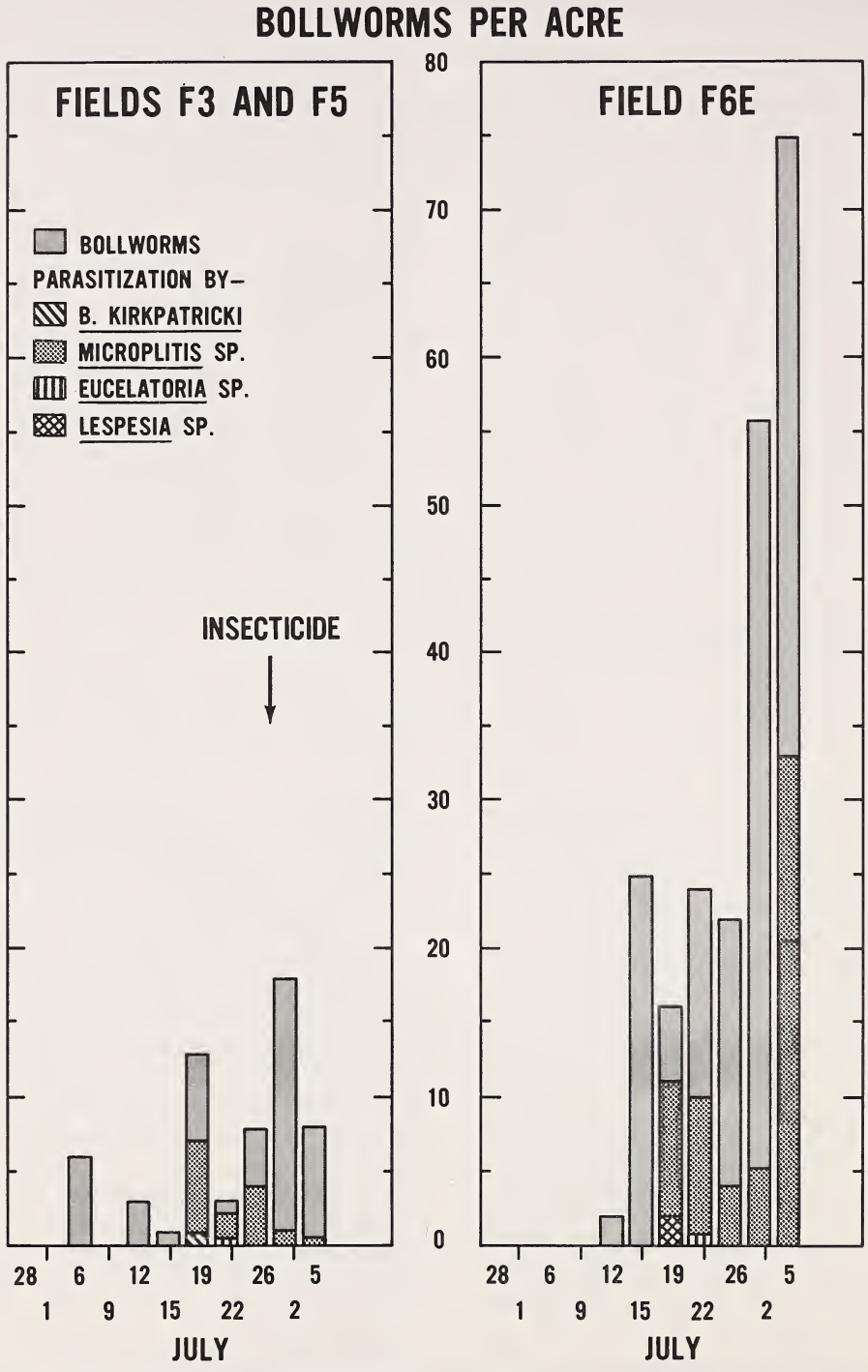

FIGURE 11.—Bollworm populations in cotton blooms with parasitization levels in fields F3, F5, and F6E, 1971

TABLE 8.—*Estimated amount of matured cotton and damage by pink bollworms and bollworms to pickable cotton, 1971*

Field and date	Green bolls	Undamaged dry bolls	Pink bollworm damage to indicated number of locules					Bollworm damage to indicated number of locules					Rot damage to indicated amount of boll		
			1	2	3	4	5	1	2	3	4	5	<½	½–¾	All
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
F3:															
Sept. 7	61.4	28.9	0.7	1.3	0.3	0.3	------	0.1	------	0.1	0.4	2.9	1.5	1.5	
13	47.7	39.1	3.0	3.2	1.2	1.1	0.6	0.1	.2	0.2	.1	.2	1.1	.8	.6
27	17.0	66.9	3.0	5.4	3.5	1.7	1.4	------	.04	.04	------	.1	------	.2	.2
F5:															
Sept. 7	50.0	43.4	.7	.5	------			.1	.1	.3	.7	2.3	.1	.9	
13	27.9	66.5	1.7	.6	------	.6	.3	------	.3	.6	------	.3	.3	.3	------
20	10.9	77.5	2.3	2.1	1.4	.4	3.2	------	.04	.04	------	.5	.2	.3	.6
F6E:															
Sept. 7	52.9	37.5	1.5	2.0	.8	.1	------	.1	.1	.1	.2	.3	2.4	1.0	.6
13	55.3	35.0	.8	1.2	.8	.7	.4	.07	.1	.4	------	.9	1.5	.8	1.2
20 and 27	27.2	58.9	3.4	3.8	1.8	.9	1.0	.09	.04	.04	.09	.9	.3	.5	1.0
F6S, Sept. 20	8.2	82.7	1.7	3.0	.8	.2	.3	.04	.2	.1	------	.5	.6	.3	.7
F6W, Sept. 27	15.6	67.3	4.7	5.9	2.3	.9	.3	.03	.1	------	.03	.7	.1	.5	1.0
F7, Sept. 20 and 27	21.4	64.5	2.6	3.3	2.5	1.1	2.6	------	.04	.2	------	.5	.2	------	.4

CONCLUSIONS

The data from the field evaluation of pink bollworm control indicate that—

(1) *B. kirkpatricki* may be effective on the square-bloom population of the pink bollworm.

(2) The early bollworm populations also may be reduced with *B. kirkpatricki.*

(3) The *C. blackburni* parasites were not introduced in adequate numbers.

(4) Both parasites warrant further field testing.

(5) The Hexalure traps may be effective in determining the timing of insecticide applications.

(6) The success of parasite introductions will be dependent on a minimized insecticide program.

(7) The sampling variance noted by Kuehl and Fye (*12*) is also applicable to the pink bollworm.

(8) Boll samples examined immediately after collection may not reveal the true extent of the pink bollworm infestation.

(9) Mortality during the egg and larval stages is extensive.

(10) Evaluating field tests is extremely difficult, especially when based on data for a single year.

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This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, beneficial insects, desirable plants, and fish or other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

U.S. GOVERNMENT PRINTING OFFICE : 1973—O-494-990